isodisomy of chromosome 17 and the first example of UPD underlying JEB-PA.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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CD44-Deficient Mice Do Not Exhibit Impairment of Epidermal Langerhans Cell Migration to Lymph Nodes after Epicutaneous Sensitization with Protein

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TO THE EDITOR

CD44 is a type I transmembrane protein that binds extracellular matrix nonsulfated glycosaminoglycan hyaluronan and has an important role in cell adhesion and migration (Isacke, 2002). Thus, CD44 is involved with leukocyte egress, tumor invasiveness, and metastasis (Isacke, 2002).

The role of CD44 in epidermal Langerhans cell (LC) migration to draining lymph nodes (LNs) was first evaluated by an antibody blocking system. Antibodies against CD44 epitopes inhibited emigration of LCs from the epidermis and prevented cultured LC binding to T-cell zones in LN-frozen sections (Weiss *et al.*, 1997). In a CD44-deficient mouse system, CD44 deficiency did not impair LC emigration from the epidermis, but significantly influenced their LN homing (Mummert *et al.*, 2004). In recent years, there has been significant progress in understanding the characteristics and kinetics of LCs. It is known that there are two kinds of Langerin$^+$ dendritic cells (DCs) (definition of LCs): one resides in the epidermis and another resides in the dermis (Bursch *et al.*, 2007). They show different migration patterns to draining LNs after immunization. Dermal Langerin$^+$ DC migration peaks early at 24 hours, whereas peak migration of epidermal LC is delayed until

Abbreviations: DC, dendritic cell; LC, Langerhans cell; LN, lymph node; Th, T helper

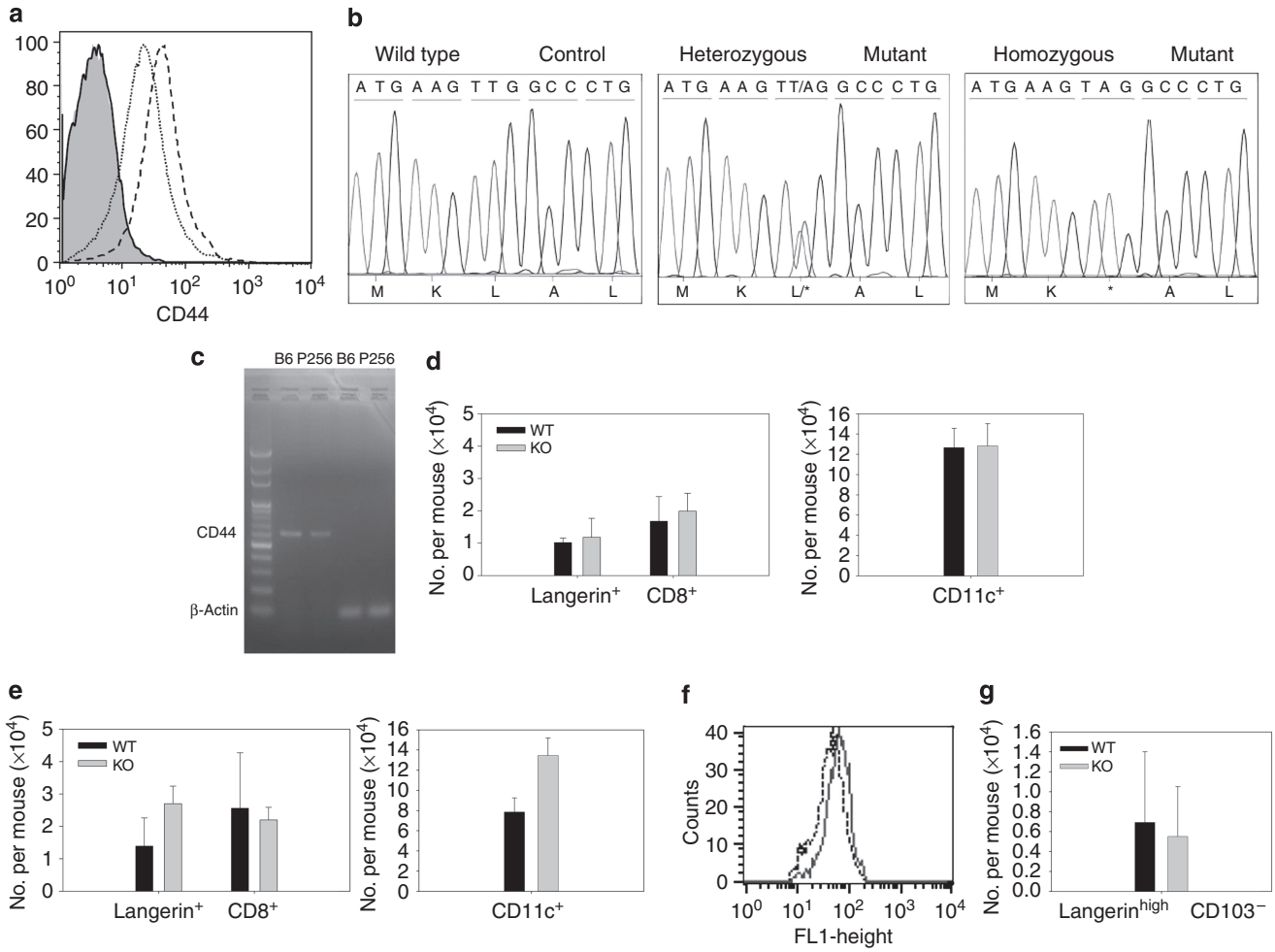

Figure 1. Numbers of dendritic cells (DCs) in draining lymph nodes (LNs). (**a**) CD44 staining of peripheral blood leucocytes of *CD44+/+* (dash), CD44*P256/+* (dotted), and *CD44P256/P256* (solid) mice, and unstained control (shaded) are shown. (**b**) All CD44 exons were subjected to DNA sequencing, and a T-to-A transversion mutation within exon 2 was found (asterisk denotes premature termination). (**c**) Reverse transcriptase-PCR amplification between exon 1 and exon 5. (**d**, **e**, **g**) Draining LNs were isolated from naive mice (**d**) or 96 hours after immunization (**e**, **g**). $CD11c^+$ cells were gated. Results are expressed as numbers per six draining LNs per mouse. The pooled data of three to five experiments are shown. (**f**) LNs were isolated from CD44-deficient mice 96 hours after patch application of ovalbumin (OVA) (dash) or OVA-DQ (solid). $Langerin^+$ cells were gated. (**g**) $Langerin^{high}$ $CD103^-$ DCs were gated.

4 days after immunization (Shklovskaya *et al.*, 2008). As previous reports failed to define LCs by specific markers and analyzed LNs for no more than 3 days, we sought to re-evaluate the migration of epidermal LCs in CD44-deficient mice using a well-established protein-patch model (Chen *et al.*, 2009). Protein antigens rather than haptens were chosen because protein antigens will cross the keratin barrier much slower, owing to their larger size and hydrophilic character, thus giving a bigger chance for epidermal LCs to capture and present them. All animal experiments were approved by the Animal Care Committee of the Medical College of National Taiwan University. Both CD44-deficient (P256) and wild-type C57BL/6 mice were from the National Mouse Mutagenesis Program Care Facility of Academia Sinica. A T to A transversion mutation within exon 2 caused a premature termination that resulted in a severely truncated 72-amino-acid CD44 protein, instead of the 778-residue wild-type CD44 protein (Figure 1a and b). Trans-splicing of mRNA between exon 1 and other parts of the standard region of CD44, which will escape the premature termination in exon 2, did not occur in P256 mice as monitored by reverse transcriptase-PCR amplification between exon 1 and exon 5 (Figure 1c).

First, the numbers of $Langerin^+$ DCs in draining LNs of naive CD44-deficient and wild-type C57BL/6 mice were examined. The numbers of $CD8^+$ DCs and total $CD11c^+$ DCs were also measured for reference. For this, an LN cell suspension was positively selected for $CD11c^+$ cells and stained with various combinations of CD11c, Langerin (intracellular), and CD8. Naive CD44-deficient and wild-type mice showed comparable numbers of $Langerin^+$ DCs, $CD8^+$ DCs, and total $CD11c^+$ DCs in their LNs (Figure 1d). Next, we examined the number of $Langerin^+$ DCs at 4 days after epicutaneous sensitization with protein antigen, which was the peak time point when epidermal LCs arrived

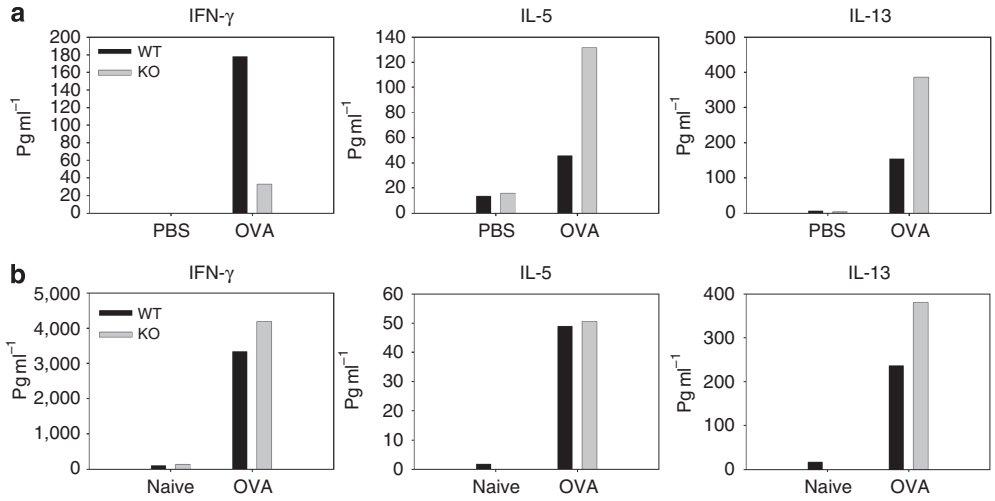

Figure 2. T-cell responses in CD44-deficient and wild-type C57BL/6 mice. Groups of CD44-deficient or wild-type mice were immunized by (**a**) patch application with ovalbumin (OVA) solution or phosphate-buffered saline control for 5 successive days or (**b**) subcutaneous injection with OVA plus complete Freund's adjuvant on day 1. At 10 days after immunization, draining lymph nodes (LNs) were isolated and *in vitro* OVA reactivation cultures were done. IFN-γ, IL-5, and IL-13 levels in 48-hour supernatants were determined by ELISA. Net concentration (concentration in the absence of OVA subtracted from the concentration in the presence of OVA) is shown. Representative results from three independent experiments are shown.

in draining LNs. As shown in Figure 1e, CD44-deficient mice had higher numbers of $CD11c^+$ DCs in draining LNs compared with wild-type mice. Wilcoxon's rank sum test was used to test the significance of difference. The *P*-value for $CD11c^+$ DC was 0.0079. The numbers of $Langerin^+$ DCs in CD44-deficient mice also seemed to be higher than those in wild-type mice. However, it did not reach statistical significance. To confirm that these $Langerin^+$ DCs were skin-derived, fluorescence-conjugated ovalbumin (OVA-DQ) was used. Figure 1f shows that $Langerin^+$ DCs in draining LNs carried this fluorescent marker. We also checked the expressions of costimulating molecules on $Langerin^+$ DCs in draining LNs at 96 hours after epicutaneous sensitization with protein antigen. $Langerin^+$ DCs in draining LNs in CD44-deficient mice showed comparable expressions of CD80, CD86, CD24, and CD40 with those in wild-type mice (data not shown). Recently, it was recognized that epidermal $Langerin^+$ DCs (LCs) and dermal $Langerin^+$ DCs could be distinguished on the basis of their expressions of CD103 (Merad *et al.*, 2008). Figure 1g shows that the numbers of $CD103^-$ $Langerin^{high}$ DCs, which indicated epidermal LCs in draining LNs 96 hours after epicutaneous immunization, were comparable in CD44-deficient and wild-type mice. The discrepancy between the results of $Langerin^+$ DCs and $CD103^-$ $Langerin^{high}$ DCs could be because of variations in $CD103^{high}$ $Langerin^{high}$ dermal DCs and $CD103^{low}$ $CD8^+$ $Langerin^{low}$ resident DCs. Taken together, these results show that CD44-deficient mice do not exhibit impairment of epidermal LC migration to LNs after epicutaneous sensitization with protein antigen.

We next checked T-cell immune responses in CD44-deficient mice using two prototypic experimental systems. Epicutaneous sensitization with protein antigen induces a predominant T helper (Th)2 response, whereas subcutaneous immunization induces a vigorous Th1 response. At 10 days after the start of immunization, drained LNs were isolated and used for *in vitro* antigen reactivation cultures. The interleukin-5 and interleukin-13 levels in supernatants of CD44-deficient mice with ovalbumin patch applications were always higher compared with those of wild-type mice (Figure 2a). However, the interferon-γ levels in supernatants of CD44-deficient mice after subcutaneous immunization with ovalbumin plus complete Freund's adjuvant (CFA) were also higher than those from wild-type mice (Figure 2b). Thus, T-cell immune responses of CD44-deficient mice are not Th1 or Th2 biased. In contrast, CD44-deficient mice showed stronger predominant Th responses after immunization.

Recently, CD44 was found to be a phagocytic receptor and involved in the resolution of noninfectious tissue inflammation by mediating the clearance of fragmented hyaluronan (Teder *et al.*, 2002; Vachon *et al.*, 2006). However, it is unlikely that these characteristics contributed to our results. First, we used gentle shaving to avoid harming the skin and applied patches without previous tape stripping or adding adjuvant. Thus, the protein-patch model that we established one decade ago examined epicutaneous sensitization under physiological, noninflammatory cutaneous conditions. Second, because fragmented hyaluronan can activate DCs, our results showing comparable expressions of costimulatory molecules on $Langerin^+$ DCs support the notion that the patched skin of CD44-deficient mice was noninflammatory. Third, CD44-deficient mice exhibit unremitting inflammation 7 days after injury, which was beyond our immunization schedule.

In summary, by using specific markers and an optimal time point, this study unexpectedly showed that CD44-deficient mice do not exhibit impairment of epidermal LC migration to LNs after epicutaneous sensitization with protein antigen.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related commentary on pg 2535

Hair Follicles from Alopecia Areata Patients Exhibit Alterations in Immune Privilege-Associated Gene Expression in Advance of Hair Loss

Journal of Investigative Dermatology (2010) **130,** 2677–2680; doi:10.1038/jid.2010.180; published online 8 July 2010

TO THE EDITOR

Alopecia areata (AA) is a putative autoimmune hair loss disease. Anagen-stage hair follicles may have immune privilege (Billingham and Silvers, 1971) comprising downregulation of major histocompatibility complex (MHC) class I and II and production of immunoregulatory agents (Paus *et al.*, 2003, 2005; Ito *et al.*, 2004). Of several hypotheses for AA pathogenesis (McElwee *et al.*, 2002; Gilhar *et al.*, 2007), one suggests that AA is a consequence of immune privilege collapse (Paus *et al.*, 2003; Ito *et al.*, 2004). Although immunohistological studies on immune privilege in AA have been performed (McDonagh *et al.*, 1993; Ito *et al.*, 2004), expression has not been objectively quantified and some immune privilege factors have not been investigated. We conducted

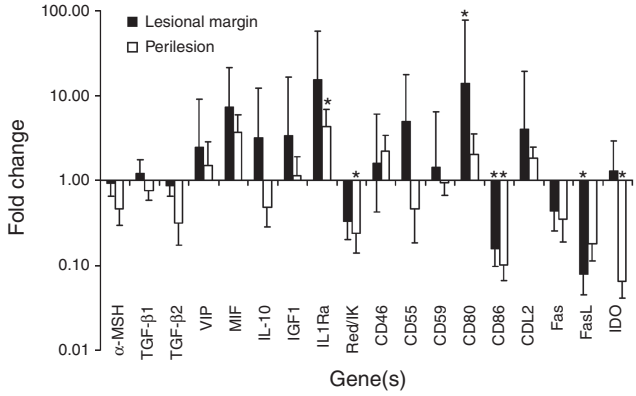

Figure 1. Differential expression of immune privilege-associated genes in the lesional margin of alopecia areata (AA)-affected patches and the perilesional scalp hair follicles as compared with normal scalp specimens. Quantitative PCR (qPCR) revealed apparent marked increased expression of IL1 receptor antagonist (IL1Ra), CD80, and macrophage inhibitory factor (MIF) in lesional margin hair follicle bulbs. Red/IK cytokine was significantly downregulated in the lesional margin. The fold change of gene expression was analyzed by qPCR and was calculated using the formula $2-\Delta\Delta Ct$, using perilesional and lesional hair follicles from four AA patients versus normal hair follicles from four controls with $\geqslant 20$ hair follicles per biopsy, and qPCR analysis was conducted in triplicate. Error bars represent the range factor difference ($2-\Delta\Delta Ct \pm \Delta Ct$ standard deviation). *Indicates $P<0.05$ by Student's *t*-test. The *y* axis is in log 10 scale.

Abbreviations: AA, alopecia areata; MHC, major histocompatibility complex